Bio QControl

P0386
SeraQ Alinity V4

REF **P0386**

The kit insert contains a detailed protocol and should be read carefully before testing the run control to ensure optimal performance

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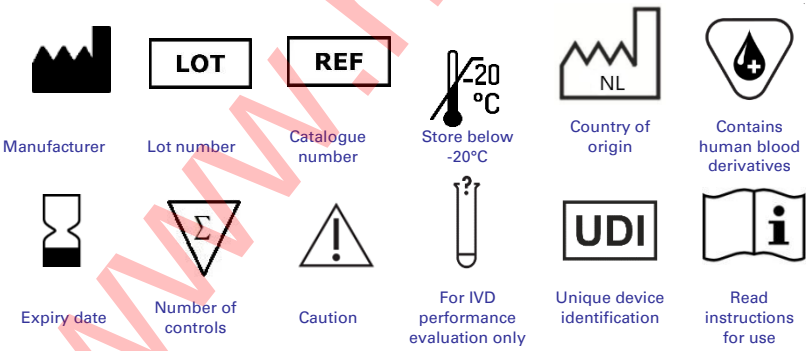
Intended Use

P0386 SeraQ Alinity V4 is intended to be used on the Abbott Alinity® platform as an external run control in combination with the assays for the detection of hepatitis B surface antigen (HBsAg), antibodies to hepatitis B core antigen (anti-HBc), antibodies to hepatitis C virus (anti-HCV), antibodies to human immunodeficiency virus types 1 and 2 (anti-HIV-1/2) and antibodies to human T-cell leukemia virus type I and II (anti-HTLV I/II) (see Table 1), performed in diagnostic and blood screening laboratories. P0386 SeraQ Alinity V4 is a multi-marker mixture of inactivated HBsAg, anti-HBc, anti-HCV, anti-HIV-1 and anti-HTLV I standards in defibrinated plasma giving a low reactive result in the Abbott Alinity® Assays. The run control is intended for repeated testing in consecutive runs of the immunoassays over time. By comparison of the sample to cut off (S/CO) values for the five markers found on P0386 SeraQ Alinity V4 one can monitor the consistent analytical sensitivity of test runs. The run control should not be used to replace internal controls or calibrators in the test kits. The test result on the run control should not be used to reject the run or delay the release of test results on donor or patient samples.

Table 1 Test kits covered by this run control

Equipment	Agent	Assays
Abbott Alinity®	Hepatitis B surface antigen (HBsAg)	Alinity s HBsAg
	Anti-hepatitis B core antigen (anti-HBc)	Alinity s Anti-HBc
	Anti-hepatitis C virus (anti-HCV)	Alinity s Anti-HCV II
	Anti-human immunodeficiency virus type 1 (anti-HIV-1)	Alinity s HIV Ag/Ab Combo
	Anti- Human T-cell leukemia virus type I (anti-HTLV I)	Alinity s HTLV I/II

Key to Symbols Used



Principle of method

A series of SeraQ multi-marker run controls have been designed for monitoring HBsAg, anti-HBc, anti-HCV, anti-HIV-1 and anti-HTLV I test performance. The run control tubes are barcoded and can be placed at random positions in sample racks of the blood screening device. The tubes are comparable in size to donor blood collection tubes. The run controls are designed to mimic naturally occurring serum specimens with low reactivity for HBsAg, anti-HBc, anti-HCV, anti-HIV-1 and anti-HTLV I. The analytical sensitivity of test kits from

different manufacturers varies and therefore for each combination of test kits a separate multi-marker run control has been designed. This SeraQ run control series includes the product P0386 SeraQ Alinity V4 for which the composition is optimised for use with the Abbott Alinity® test system. The P0386 SeraQ Alinity V4 run control is designed to generate assay response values (i.e. S/CO ratios) positioned in the low positive range of the assays. Routine use of external run controls enables laboratories to monitor day-to-day test performance and IVD reagent lot variation.

Traceability of antigen and antibody concentrations

For each HBsAg, anti-HBc, anti-HCV, anti-HIV-1 and anti-HTLV-I an internal serum standard has been established[1] from which reference panels and run controls are prepared by gravimetrically recorded dilution steps. The undiluted secondary standard for HBsAg is derived from the same purified heat-inactivated source material as is used for preparation of the 2nd WHO HBsAg adw2 (00/588) International Standard (IS)[1-4]. Studies with the later established WHO international hepatitis B virus genotype reference panel showed that the heat-inactivation of HBsAg in the International Standard had little impact on the detectability in immuno-assays[4]. The HBsAg concentration in the run control has been set at 0.088 IU/mL[1]. One IU of heat-inactivated HBsAg was found to be equivalent to 0.67 ng HBsAg when historically calibrated against the first WHO standard established by the Paul Ehrlich Institute[1,2], comparable to conversion factors of 0.58 and 0.71 reported in WHO collaborative studies[3,4]. No unitage could be assigned to the internal standards for anti-HBc, anti-HCV, anti-HIV-1 and anti-HTLV-I since international reference preparations are not available. The consistent concentration of the analytes in consecutive SeraQ run control batches is guaranteed by release testing against a reference batch of the run control kept frozen at -30°C. These reference batches are derived from the same undiluted internal standards that are used for manufacturing of the SeraQ run controls.

Materials Provided

The run control contains 3.0 mL of P0386 SeraQ Alinity V3 run control and 0.01% (w/v) Thimerosal as preservative and is provided in two formats as detailed in Table 2.

Table 2. Description of kit formats and contents

Cat. Code	UDI code	Quantity run control	Size tubes	Secondary packing
P0386/01	8718719830194	60 x 3.0 mL	10 mL	60 tube rack in box
P0386/02	8718719830195	10 x 3.0 mL	10 mL	10 tubes in box

Materials not provided

Pipettes or pipetting devices for use in IVD test systems. Vortex instrument for thorough mixing of samples prior to use.

Storage Instructions

Store unopened tubes at or below -20°C. For each Alinity instrument thaw one run control tube in a water bath of 37°C until ice clot has disappeared. After thawing, the run control tubes should be stored at 2°C to 8°C for no longer than one week.

Warning and precautions

P0386 SeraQ Alinity V4 run controls are prepared from serum standards, in which virus has been inactivated by validated methods applied in the plasma industry[1]. Infectivity and inactivation data have been analysed to demonstrate absence of residual infectivity of

HBV, HCV, HIV-1 and HTLV-I in the run controls[1]. The serum matrix in the run controls has been tested for infectious disease markers by serologic and molecular screening methods. However, no screening procedure can offer complete assurance that products derived from human blood cannot transmit undetected infectious agents. The run control should only be used by trained laboratory workers who are aware of the potential risk of infectious agents in human serum samples and take the necessary precautions.

- SeraQ run controls should be handled with the normal preventive measures in a serology laboratory[5,6].
- This product contains human plasma and traces of biological source material of non-human origin (bovine thrombin).
- The use of the run control in other assay configurations should be avoided and is not supported by the manufacturer.
- Wear disposable gloves when handling samples.
- Do not eat drink, smoke or apply cosmetics in areas where specimens are handled.
- Do not pipette by mouth.
- If skin or mucous membrane exposure occurs, immediately wash the area with copious amounts of water.
- Disinfect spills using a 0.5% hypochlorite solution (1:10 v/v household bleach) or equivalent disinfectant.
- Dispose unused or spilled materials according to the normal practices for biological waste disposal in your institution.
- If precipitates are visible, mix the run controls for 2 minutes thoroughly using a vortex instrument.
- Do not use run controls beyond one-week storage at 2-8°C.
- Store run controls in an upright position.
- Validation of the diagnostic test results must be based on the specifications set by the manufacturer of the test kit and not be influenced by the test result on the run control.

Reagent preparation

- For first use of the run control thaw the tube quickly in a water bath at 37°C.
- Mix gently during thawing until contents are just thawed
- Immediately after ice clot has disappeared remove the run control tube from the water bath.
- Before testing allow the run control tube to adapt to room temperature.
- Mix the run control tube thoroughly prior to use with a vortex instrument.
- Place the run control tube at the specified positions in the sample racks of the Alinity system for regular donor or patient samples.
- Test on the Abbott Alinity platform with the assays mentioned in Table 1 according to the manufacturer's instructions.
- Store the opened tube immediately after use at 2-8 °C (see storage instructions).

Expected assay response values

The expected results for the P0386 Abbott Alinity V4 run control are as follows:

1. HBsAg range S/CO ratio: 2.2 – 3.6
2. Anti-HBc range S/CO ratio: 2.2 – 3.6
3. anti-HCV range S/CO ratio: 2.2 – 4.0
4. anti-HIV range S/CO ratio: 1.7 – 4.2
5. anti-HTLV I range S/CO ratio: 1.9 – 2.7

Each Alinity reagent lot appears to have its own dose response curve and distribution of S/CO values on SeraQ run controls. This depends on the analytical sensitivity of the

Abbott Alinity reagent lots that are in use. Thus, it cannot be guaranteed that the assay response values will always fall within these ranges. P0386 SeraQ Alinity V4 run control serves as an independent standard for monitoring consistent analytical sensitivity of Abbott Alinity reagent lots over time.

Interpretation of Results

Calculations

Subsequent test runs can be analysed by appropriate statistical approaches on the S/CO ratios obtained on the external control samples.

Assay response values

To obtain the test kit batch specific reference values for each marker, an initial collection of at least 20 consecutive test results is required. Upon collecting additional data, the chart characteristics may be updated.

- The S/CO values for HBsAg, anti-HCV and anti-HIV are 'log normally' distributed. For the Abbott Alinity assays one should use the logarithm of S/CO ratios for calculation of the geometric mean and confidence interval.
  - Calculate from each measurement the log S/CO value.
  - Calculate average and standard deviation on this log transformed values; log (Average) and log (Standard Deviation).
  - Calculate the (geometric) mean in S/CO ratio by taking the anti-log value of the log (Average)Use Table 3 to obtain Student-t-values belonging to the 95% and 99% CI for different number of observations (n)
  - Calculate the log(95% and 99% CI) as follows:

    Log (99% Lower limit): log (Average) – (99%) Student-t-Value x log (Standard Deviation)
    Log (95% Lower limit): log (Average) – (95%) Student-t-Value x log (Standard Deviation)
    Log (95% Upper limit): log (Average) + (95%) Student-t-Value x log (Standard Deviation)
    Log (99% Upper limit): log (Average) + (99%) Student-t-Value x log (Standard Deviation)
  - Take the anti-log values for calculating the confidence limits in S/CO ratio. To visualize the individual S/CO values make a Levey-Jennings control chart on a linear scale. S/CO ratios plotted on a linear scale depict the upper 95% and 99% confidence limits at greater distance from the geometric mean S/CO value than the lower confidence limits (see example Figure 1).

Levey-Jennings chart

The Levey-Jennings chart is a graph in which quality control results are plotted over subsequent test runs in time to give a visual indication when a laboratory test is (not) working well. The data points for each test run in the scatter plot below (see Figure 1a-e) show the distance from the geometric mean S/CO ratio (green line in graph) which is the expected response level for the run control. The orange and red lines represent the 95% and 99% CI respectively. The data represents individual measurements of six instruments.

Table 3. Relation of Student t value and numbers of runs (n) to calculate confidence intervals.

Runs (n)	t-value at 95% C.I.	t-value at 99% C.I.
10	2.306	3.355
20	2.101	2.878
30	2.048	2.763
Infinite	1.960	2.576

Infinite equals the normal distribution

Figure 1. Levey-Jennings charts of P0386 SeraQ Alinity V4 run control results in Abbott Alinity S assays from two laboratories (represented by the orange and blue dots) using different Alinity instruments. The average (green line) and 95% and 99% CI (orange and red lines) are log transformed as explained in the text.

Figure 1a. Abbott Alinity s HBsAg assay.

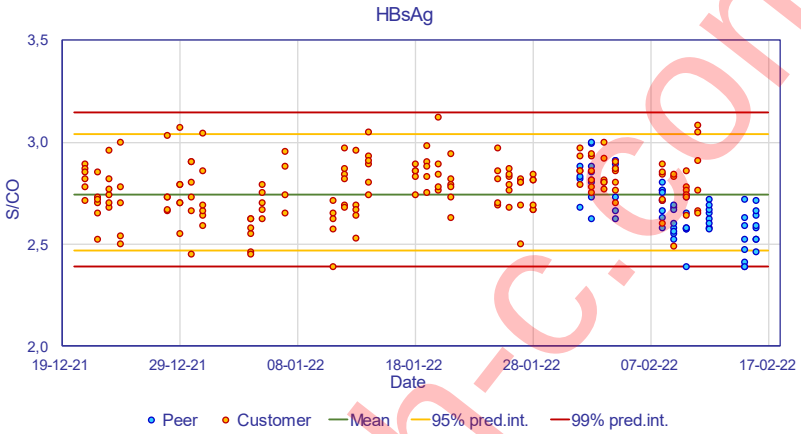


Figure 1b. Abbott Alinity s anti-HBc assay.

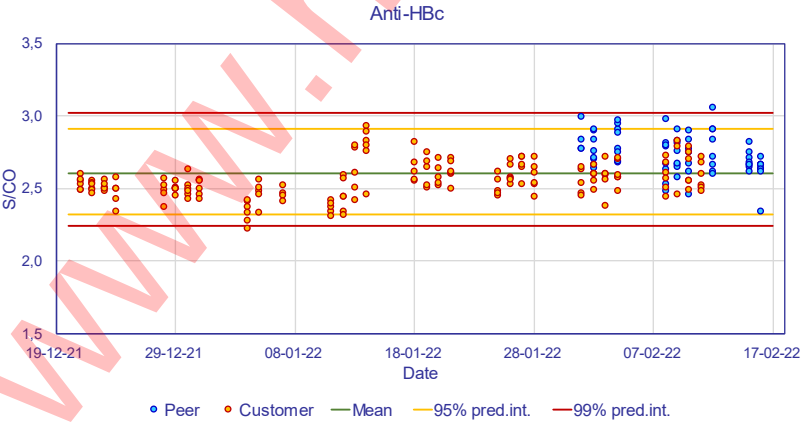


Figure 1c. Abbott Alinity s anti-HCVII assay.

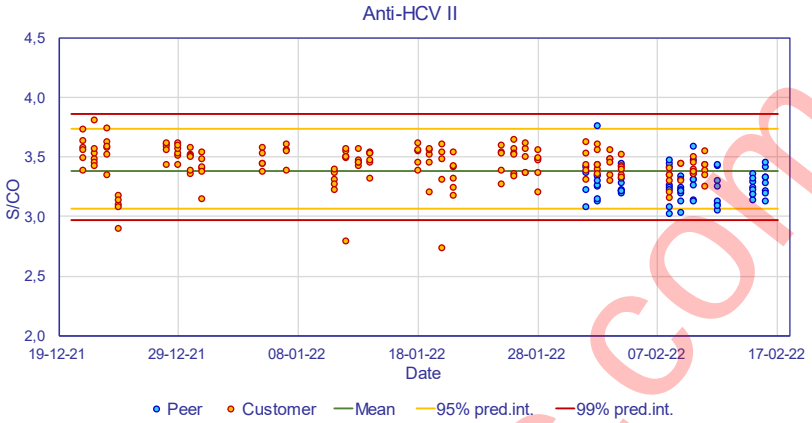


Figure 1d. Abbott Alinity s HIV-Ag/Ab Combo assay

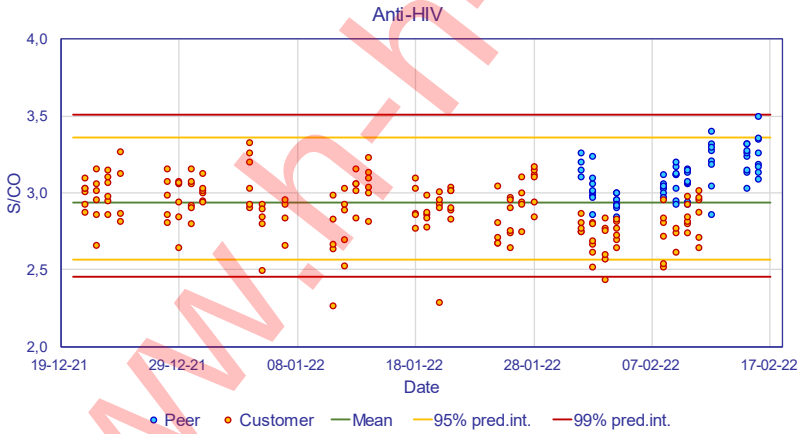
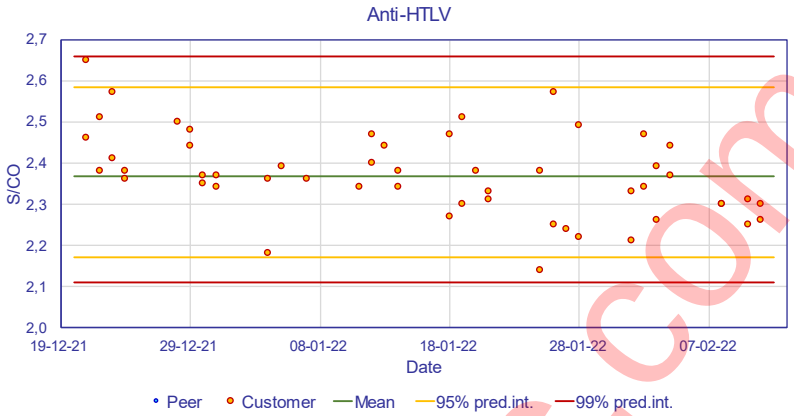


Figure 1e. Abbott Alinity s Anti-HTLV I/II assay



Interpretation

Knowing the 95% and 99% CI for generating a Levey-Jennings chart one can use Westgard rules[7] to interpret values outside the predictive limits for identifying trends and aberrant results. One can find guidance on how to identify trends and outliers on the website www.westgard.com.

- Negative or positive trends resulting from gradual changes in test performance and not reported by the internal kit controls and/or alert systems in the test robot, are indicative for a lack of maintenance, the need for recalibration of equipment, or degradation of reagents. These are systematic errors. In case a trend is recognised, the laboratory is encouraged to identify the root cause of the deviation.
- Aberrant results like a negative response on the run control or a result outside the 99% CI are indicative for incidental errors that need further investigation to identify the root cause. The identification of the root cause of aberrant results is beyond the scope of the intended use of the run controls.
- Differences between S/CO values of laboratories could be attributed to different reagent lots or run control batches that are in use.

Analytical Performance Characteristics

SeraQ run controls have been designed by examination of the response curves on dilutions of the internal standards and as such relate to the analytical sensitivity of immunoassays. In the following paragraph the essential analytical performance characteristics of SeraQ run controls are presented.

Dose response and analytical sensitivity

By analysing standard dilution series the relationship between S/CO values and concentration of the analyte can be established[8,9]. Plotting (transformed) S/CO values against lLog concentration of analyte using linear regression analysis enables calculation of correlation coefficients. It is recommended to use a transformation resulting in an optimal correlation. Figures 2a-e show linear dose response relations in the Abbott Alinity HBsAg, anti-HBc, anti-HCVII, HIV-Ag/Ab Combo and Anti-HTLV I/II Assays obtained after log transformation of dilution factor and S/CO values.

Figure 2a. Dose response in Abbott Alinity s HBsAg assay. Log HBsAg S/CO values are plotted against log dilution of HBsAg standard (r^2=0.99).

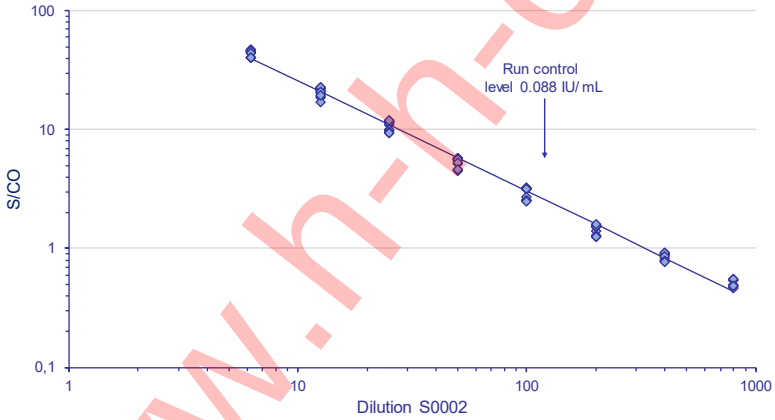


Figure 2b. Dose response in Abbott Alinity s anti-HBc assay. Log anti-HBc S/CO values are plotted aginst log dilution of anti-HBc standard (r^2=0.99).

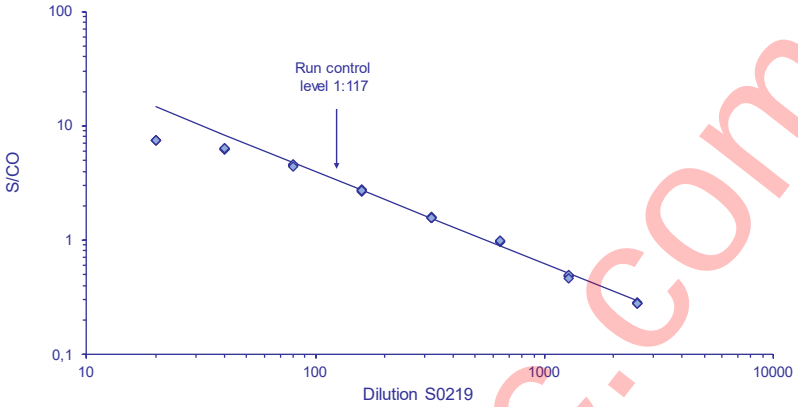


Figure 2c. Dose response in Abbott Alinity s anti-HCV II assay. Log anti-HCV S/CO values are plotted aginst log dilution of anti-HCV standard (r^2=0.99).

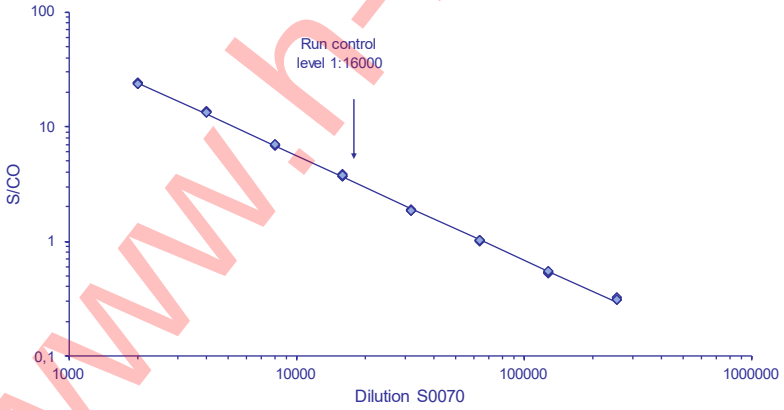


Figure 2d. Dose response in Abbott Alinity s HIV Ag/Ab Combo assay. Log anti-HIV-1 S/CO values are plotted aginst log dilution of anti-HIV-1 standard (r^2=0.99).

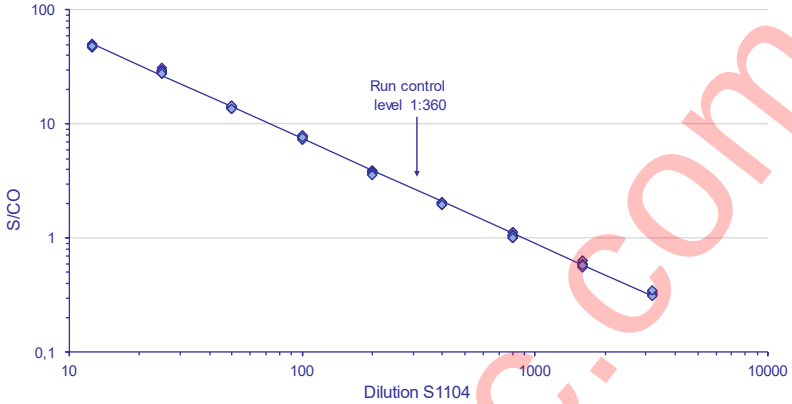
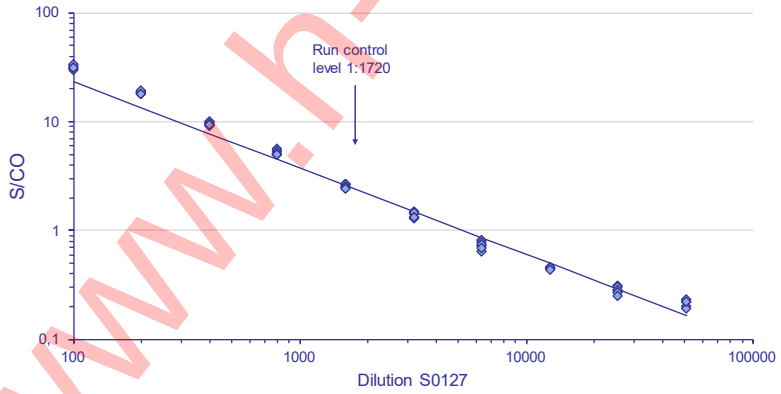


Figure 2e. Dose response in Abbott Alinity s HLTV I/II assay. Log anti-HTLV-I S/CO values are plotted aginst log dilution of anti-HTLV-I standard (r^2=0.99).



Abbott Alinity Assay response values on P0386 SeraQ Alinity V4 run control
Table 4 gives an example of results obtained with a few Alinity reagent lots in two laboratories.

Table 4. Abbott Alinity Assay response values on P0386 SeraQ Alinity V4 run control

Alinity Assay	n	geomean S/CO	95% CI* S/CO	99% CI* S/CO
HBsAg	237	2.74	2.48 – 3.02	2.41 - 3.12
Anti-HBc	238^	2.60^	2.37 – 2.86	2.30 – 2.95
Anti-HCV II	232	3.39	3.07 – 3.74	2.97 - 3.86
HIV Ab/Ag	236	2.94	2.56 – 3.36	2.46 – 3.51
Anti-HTLV-I	210	2.21	1.92 – 2.55	1.83 – 2.66

*for calculation see instructions above. . ^in another study a third laboratory reported a geomean (range) anti-HBc S/CO value (n=68) of 3.17 (2.96-3.56).

Variation in immune-assay reagent lots
Variation in S/CO ratio on run controls reflects the difference in analytical sensitivity of assay runs and reagent lots. Different batches of SeraQ run controls are prepared from the same standards. Therefore the composition of the multi-marker run controls is consistent from batch to batch. This is confirmed by multi-variance analysis on large data sets of the previously used PRISM Assay showing that reagent lots are the major source of variation in S/CO values. Table 5 shows an example of the S/CO response values in different Abbott Alinity anti-HCV reagent lots on two SeraQ run control batches used by two laboratories. Similar results were observed for other serologic assays.

Table 5. Geomean anti-HCV S/CO ratios and %CV in different Alinity reagent lots on two batches of P0386 SeraQ Alinity V4 run control used by two laboratories.

Lab	P0386 SeraQ run control batch	Anti-HCV Alinity Reagent Lot	Start	End	n	Geo Mean S/CO	%CV
1	B4355-004	28510BE00	21-12-21	31-12-21	46	3,47	5,41
	B4355-004	30719BE00	05-01-22	03-02-22	77	3,46	3,27
	B4355-004	34331BE00	01-02-22	11-02-22	39	3,40	2,70
2	B4355-005	34331BE00	01-02-22	16-02-22	68	3,27	4,06

Limitations

- SeraQ run controls were designed for monitoring the analytical performance of IVD kits. They cannot be used to evaluate the diagnostic sensitivity of IVD kits.
- The run control must not be substituted for the mandatory controls or calibrators provided with IVD test kits for calculating the cut off and/or criteria for releasing test results.
- The response values on the run controls should not be used to release or reject the test run but can be used as an aid in the assessment of analytical performance.
- The expected S/CO values and 99% predictive intervals have been established with a limited number of Alinity reagent lots. It cannot be guaranteed that S/CO values obtained with new reagent lots will always fall within these limits.

References

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